NASA-CR-204805

Reprinted from
ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Volume 200 Pages 73-93
December 29, 1972

ELECTRON MICROSCOPIC FINDINGS IN THE LUNGS OF MINERS

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To our knowledge, electron microscopic studies of the lungs of coal miners in the United States have not previously been reported. The purpose of this communication is to report our findings, as determined by electron microscopy, in a study of the lungs of men exposed to coal dust.

METHODS *

Tissue was obtained from the lungs of four subjects who had been exposed to bituminous coal dust in the course of their work. Three had been mainly underground coal miners; the fourth worked as a boiler repairman at a power plant that burned bituminous coal. Details regarding age, number of years' exposure, and job classification, insofar as they could be determined, are presented in TABLE 1. In the case of each underground coal miner, the tissue was obtained at autopsy from an unfixed lung. Lung tissue from the boiler repairman was obtained by surgical biopsy at the time of an operation to replace a stenosed mitral valve, which had resulted from rheumatic fever at age 13. One portion of each tissue specimen was immediately placed in 7% collidine buffered glutaraldehyde solution for examination by the electron microscope. Another portion was placed in formalin solution and processed by routine methods for histologic examination by light microscopy in the Department of Pathology at West Virginia University Medical Center, Morgantown, West Virginia.

The specimen in the glutaraldehyde solution to be examined by electron microscopy was air-mailed to California, where the tissue was post-fixed in 1% osmium tetroxide for one hour, dehydrated in graded acetone, and embedded in Araldite® 502 resin. Sections were cut from the cured blocks with a diamond knife on a Porter-Blum I microtome, picked up on uncoated grids, and stained first with lead and then with uranyl acetate. These grids were then examined with a Philips 300 electron microscope.

When possible, a chest x-ray taken prior to the time of obtaining lung tissue was selected; photographs of these x-rays are included in FIGURE 1a–d.

Pulmonary function studies were performed on two of the subjects. For

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subject 3, the studies were performed three years prior to his death; and for subject 4, 16 months after the lung biopsy was obtained. Details of the methods used in our laboratory have been reported elsewhere.[1,2]

FINDINGS

Brief summaries of the clinical course of each of the subjects whose lung tissue was examined are presented in the following paragraphs for the purpose of correlation with pathological findings.

Case 1

This man was a 60-year-old former coal miner, who had worked at that occupation for 40 years. He had spent the major part of his working time underground at the coal face, although he had worked on the surface at the tipple for an indeterminant period. He had smoked 10 to 15 cigarettes per day for 40 years. He had several years' history of chronic cough, wheezing, and dyspnea on exertion. A chest radiograph made elsewhere in 1968 was said to show evidence of "silicosis." An admission chest radiograph at West Virginia University Hospital, Morgantown, West Virginia, showed a pattern of large and small opacities consistent with Stage A, complicated on a background of category 3/3 simple coal workers' pneumoconiosis.[3]

He died at West Virginia University Hospital as a result of uncontrollable hemorrhage at the site of an aorto-femoral bypass graft performed because of an acute occlusion of the right femoral artery. The lung tissue was obtained at autopsy performed within two hours of death.

Light microscopy of the specimen stained with hematoxylin and eosin revealed mild changes of perivascular fibrosis. Some vessels were occluded by organized thrombi, and there was increased fibrous tissue of the pleural surface. Some crystals, thought to be silica, were seen inside the fibrotic scars. Focal areas of interstitial fibrosis and moderate degrees of anthrocotic pigment in the septum and the pleural surface were noted (FIGURE 2a).

Electron microscopy showed a moderately thickened basement membrane, often containing fibrous material in the alveolar-capillary wall. In most areas the endothelial lining of the capillaries was intact, although frequently vesiculated. The epithelial lining of the alveolar sacs was usually damaged, disrupted, or entirely absent (FIGURE 3a). Since this finding was common to all three autopsy specimens, but not to the biopsy material, it was assumed that this was a postmortem artifact. The destruction and vesiculation seen in many cells, including the vesiculated endothelial cells mentioned above, may have occurred in the same way.

Elastic tissue appeared in abundance in the interstitial areas among the collagen fibers and in the alveolar septae (FIGURE 3a). Elastic fibers appeared in the other cases, also, but not in such quantity.

Throughout the areas studied, a great quantity of collagen type connective tissue was noted. It was in this kind of tissue that cells containing particulate material were most often found.

Four types of particles were common to all four lungs: needle-shaped, angular, rectangular layered, and large aggregates (TABLE 4). The needle-

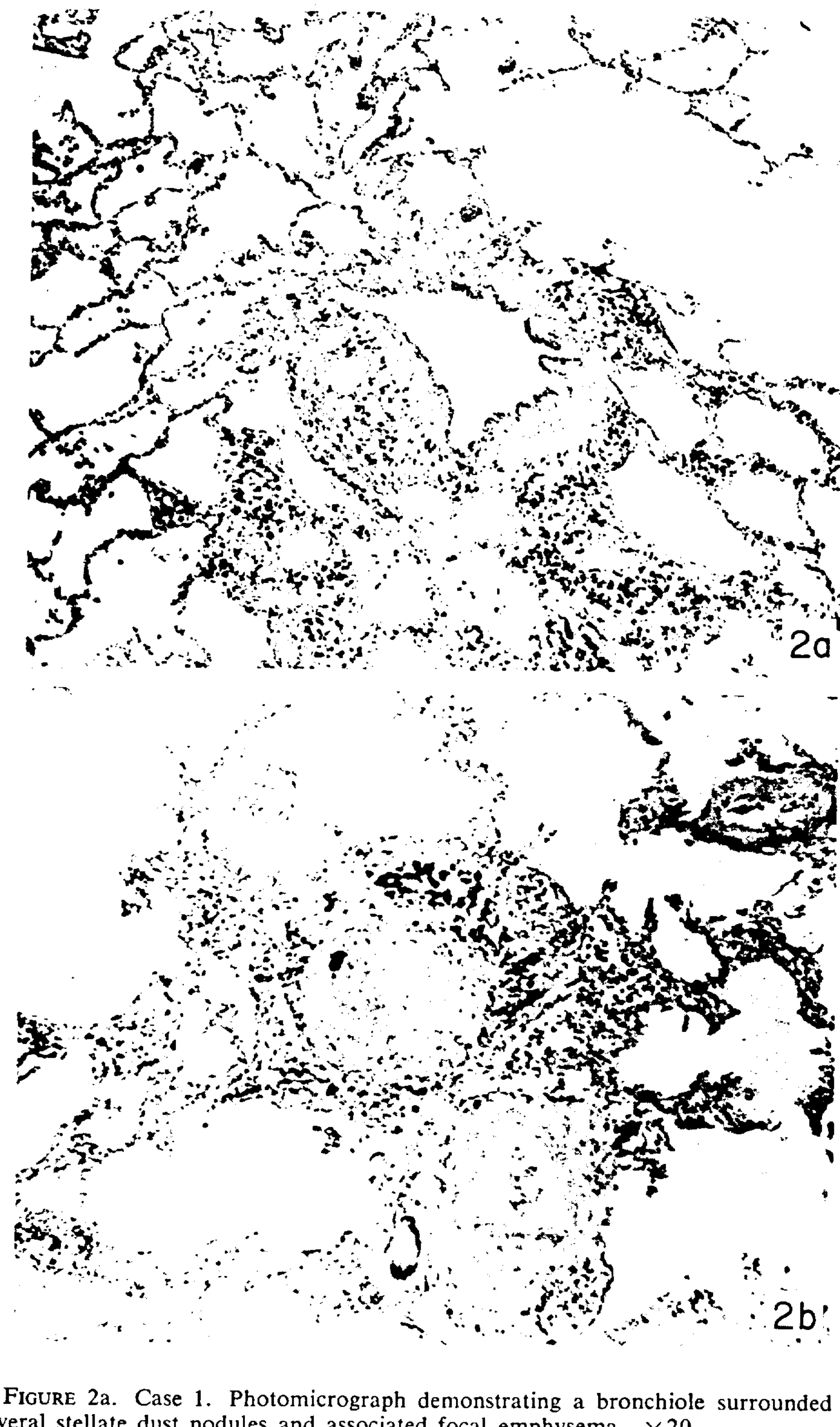

FIGURE 2a. Case 1. Photomicrograph demonstrating a bronchiole surrounded by several stellate dust nodules and associated focal emphysema. $\times 20$.
FIGURE 2b. Case 2. Photomicrograph demonstrating a hyalinized dust nodule containing pigment in the periphery, an adjacent arteriole, and surrounding focal emphysema. $\times 32$.

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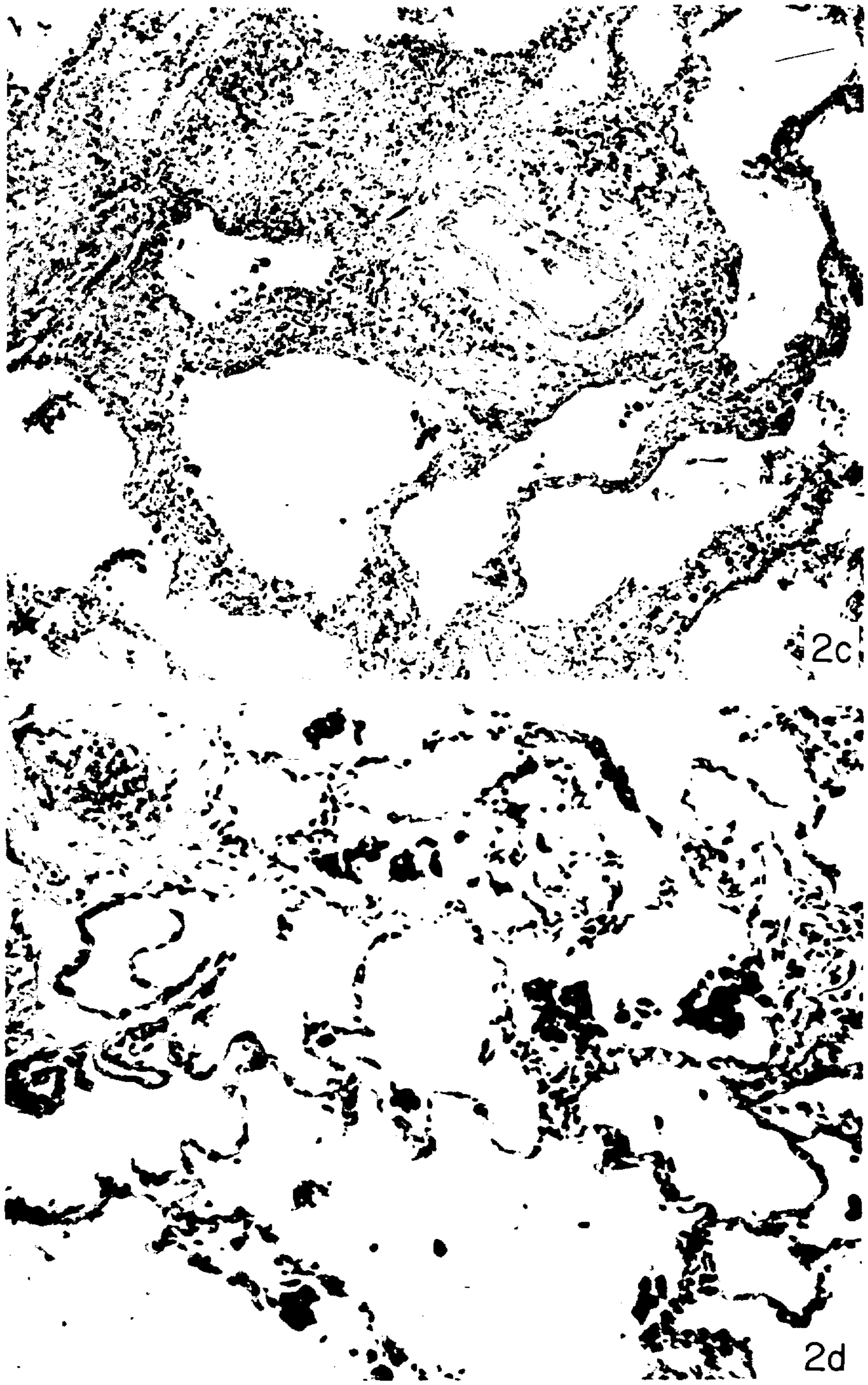
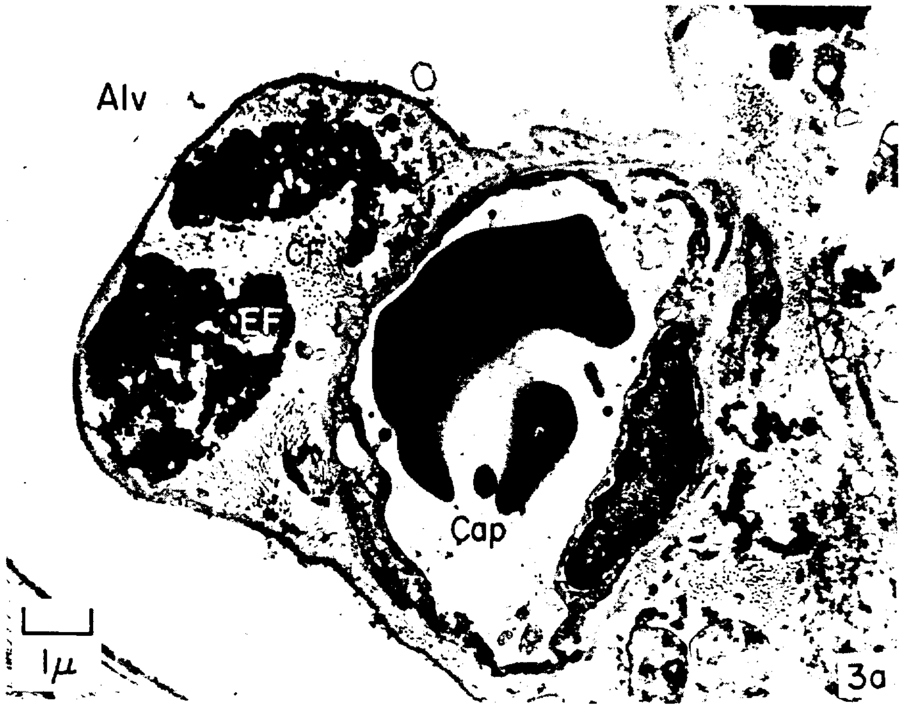


FIGURE 2c. Case 3. Photomicrograph demonstrating an arteriole surrounded by conglomerations of dust nodules, abnormally thickened alveolar remnants, and associated focal emphysema. ×32.

FIGURE 2d. Case 4. Photomicrograph demonstrating small arteriole, upper left, with some surrounding fibrosis. Pigment laden macrophages are evident within alveolar spaces whose walls are intact and not abnormally thickened. ×32.



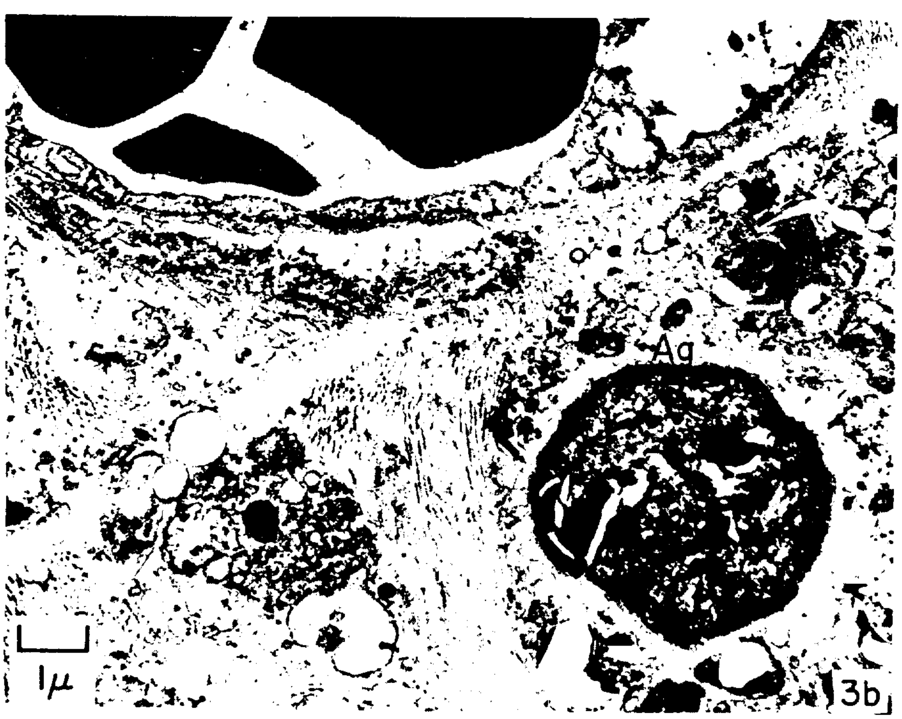


FIGURE 3a. Case 1. Alveolo-capillary wall, showing absence of epithelial cell layer and presence of elastic fibers (EF) and collagen (CF) in the basement membrane. ×8,250.

FIGURE 3b. Case 1. Small blood vessel showing proximity of debris-containing cell. Large granular aggregate (Ag). ×8,250.

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shaped particles, if small (1 μ or less), were usually found at the periphery of cytosomes. Larger needles were seen either in cytosomes or free in the cytoplasm (FIGURE 4a). They were composed of a dense, hard material that was difficult to cut and very often tore the sections. Material similar to this was described by Schulz [4] and considered to be a silicate of magnesium or iron. In the study by Schulz, the needles were seen in a sandblaster's lung.

A second type of particle was a moderately dense, homogeneous(smooth) appearing particle, sometimes having a dense border (FIGURE 4b). Some of these particles were observed in cytosomes, some free in the cytoplasm, and some in the large aggregates to be described later. These angular particles resemble those described by Schulz [4] from a "biscuit grinder's" lung (Carborundum lung) and were determined to be either silicon carbonate (SiC) or Aluminum oxide (Al_2O_3).

Another type of material appeared as rectangular, moderately to heavily dense particles with a cross striation caused either by overlapping or, more probably, from "chatter" created when sectioning this dense material. This type of particle was observed in the cytoplasm (FIGURE 4a). These particles may be dust, as described by Schulz [4] and classified mainly as carbon and quartz.

The similarities of these particles to published descriptions are noted only as an indication of their possible constitution, not as positive identification.

The fourth type of deposit common to all four lungs was a large aggregate (3–4 μ) of granular material sometimes containing needles and/or angular particles or other debris (FIGURE 3b & 4b). The granular material appeared denser at the periphery, and no membrane was seen surrounding these aggregates. The granular material could be seen scattered in small clumps in the cytoplasm, in some of the cytosomes of dust containing cells in the connective tissue, and in macrophages in the alveolar spaces. In some sections there were cells containing particles intimately associated with small blood vessels. They were embedded in the connective tissue directly adjacent to the basement lamina of the blood vessel (FIGURE 3b).

Case 2

This 64-year-old male had worked as an underground coal miner for approximately 45 years, mainly at the face. He had smoked 20 to 30 cigarettes per day for nearly 20 years, having stopped in 1942. He was treated in 1969 for tuberculous infection of the right shoulder. His terminal admission to West Virginia University Hospital in January, 1970, was for respiratory distress associated with a mass in the right infrahilar area and scattered smaller masses in both lungs. The patient died shortly after admission of what was shown at autopsy to be undifferentiated bronchogenic carcinoma of the right lower lobe with widespread metastases. The chest radiograph made prior to the appearance of the lung tumor showed a pattern of large and small opacities consistent with Stage A, complicated on a background of category 2/3 simple coal workers' pneumoconiosis. Tissue was obtained from the noncancerous lung within approximately two hours after death.

Light microscopy of the cancerous lung revealed poorly differentiated bronchogenic carcinoma arising from the right lower lobe bronchus with metastases to the hilar lymph nodes and throughout the lung parenchyma. Other portions of the lungs showed numerous small nodules, the central portions of which

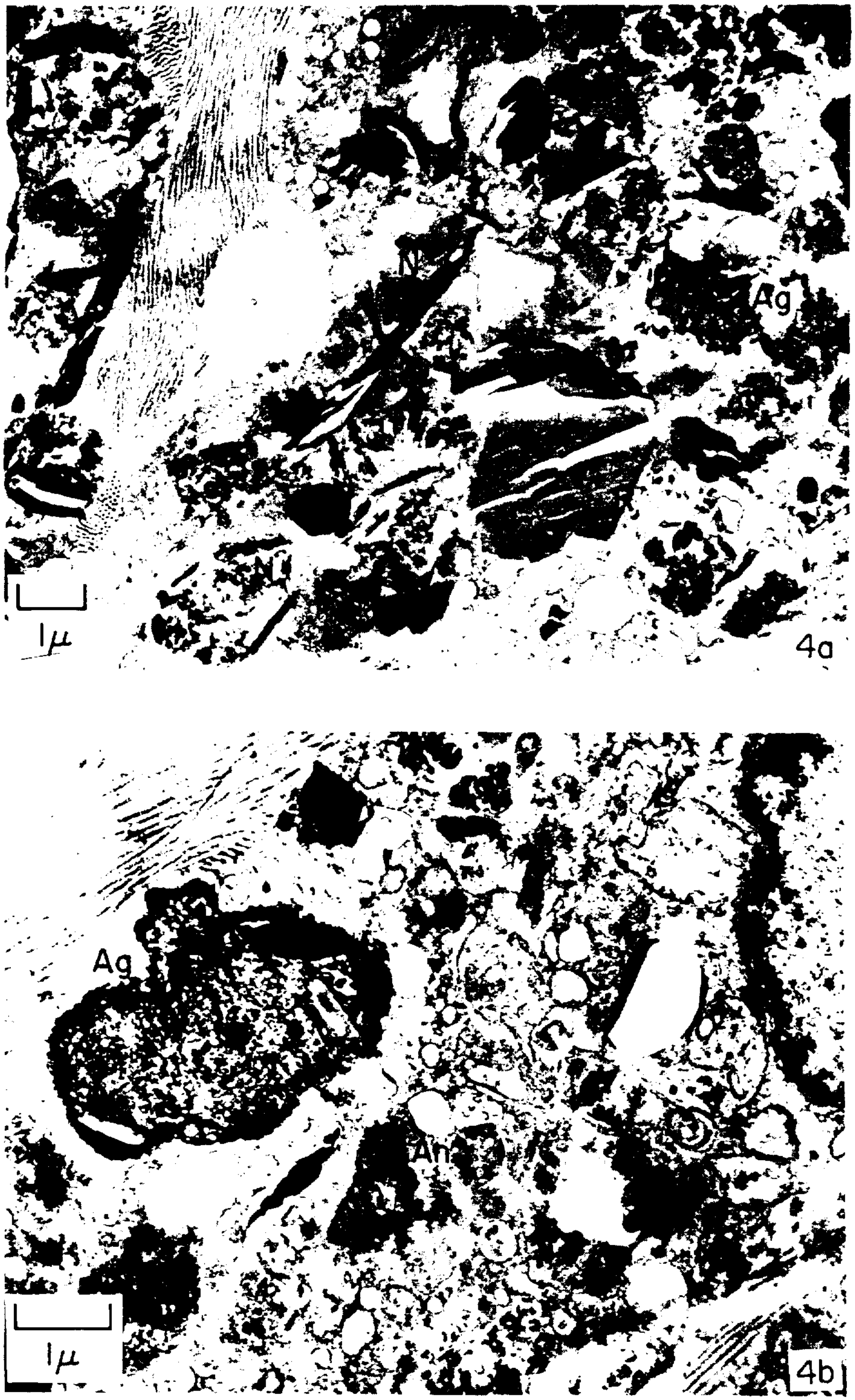

FIGURE 4a. Case 1. Area showing all four types of particles: needles (N), angular (An), rectangular (R), and aggregates (Ag). ×8,250.

FIGURE 4b. Case 1. Area showing large aggregate and angular particles with dense borders. ×12,400.

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were fibrotic and hyalinized, occasionally showing central necrosis. Peripheral parts of the nodules showed abundant carbon pigment and crystalline granules that polarized like silica at the periphery of the nodules (FIGURE 2b).

Electron microscopic examination showed the alveolo-capillary walls to be in a deteriorated condition. The basement membranes were thickened and often contained much fibrillar material. Some of this material was collagen, revealing the characteristic striations, and some was a "wooly," textured material. As in the previous case, the endothelium of the capillaries was intact but showed vesiculation and other postmortem changes, and the epithelial layer was damaged or, more often, absent (FIGURE 5a).

An abundance of elastic tissue was present in the sections viewed, although not so much as in those from Subject 1. Collagen fibers were also prominent in interstitial areas, in alveolar septae, and in basement membranes of capillaries. The interstitial collagen fibers in some areas appeared in whorl-like arrays, while other areas of fibers were less well-organized in arrangement (FIGURE 7b).

Within the fibrillar material were areas of small mononuclear cells. In the same area there were many cells containing various types of particles. This lung contained the widest variety of particulate matter of all the material studied (TABLE 4). There was needle-like material lining the periphery of cytosomes (FIGURE 5b). Some of this material showed overlapping of the needles (FIGURE 6a). Some of what seemed to be the same type of material appeared as long rectangles rather than as needles, (FIGURE 5b) and one area showed one very large ($5\ \mu \times 0.5\ \mu$) longitudinally-striated piece of debris and one smaller piece ($2\ \mu \times 0.15\ \mu$). Both appeared to have cytosomes attached to them. In both cases the particles were too large to be engulfed by the cytosomes.

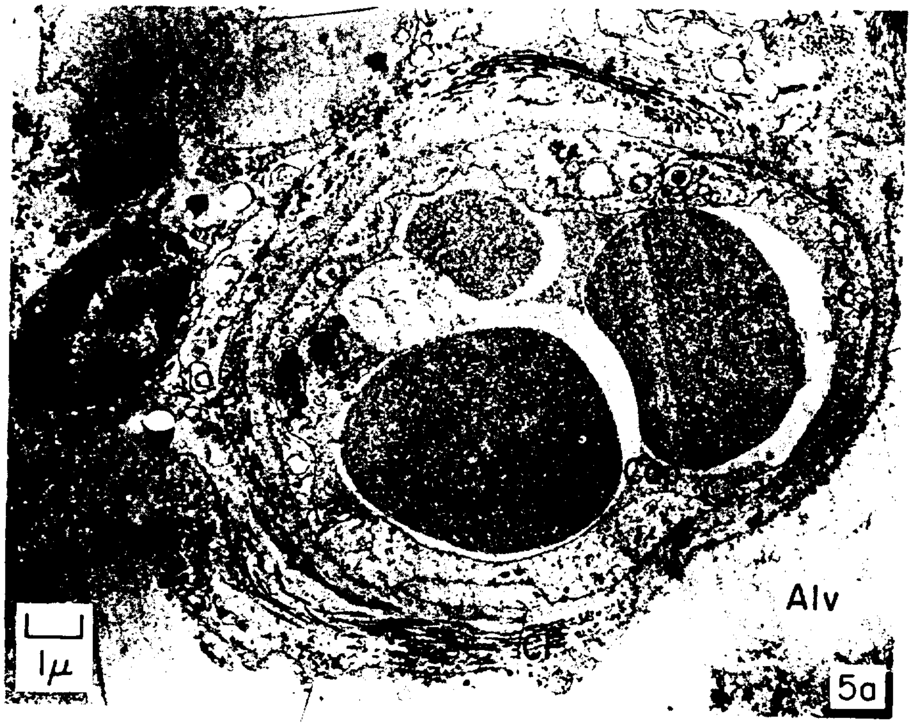
Only a few angular, homogeneous particles were noted in this lung (FIGURE 7b), but two other types of particles did appear. One was always seen in the center of membrane-bound vesicles. It was a small squarish piece ($0.5\ \mu$) of high electron density (FIGURE 7a). The other type of particle was comprised of round, often perfect spheres that resembled the spheres of silicon dioxide visualized by Schulz (FIGURE 7b).

The larger, rectangular particles showing horizontal striping or chatter were also seen here (FIGURE 6a), as were the large granular aggregates (FIGURE 6b). Some of the aggregates contained only granules, while some contained needles and other debris in addition to the granular material. This material was also seen, less densely packed, in the cytosomes.

Case 3

This 51-year-old male had worked as a roof bolter for 20 years. He had smoked approximately 30 cigarettes per day for 27 years, having stopped about two years prior to his first admission to West Virginia University Medical Center in 1967. At the time of admission, he complained of dyspnea after climbing one flight of stairs or walking at an ordinary pace uphill. The duration of the dyspnea was two years. He also complained of chronic cough, productive of one to two tablespoonsful of phlegm, and recurrent "colds" that settled in the chest and were associated with purulent sputum. The latter symptoms had been present at least two years.

A chest film obtained on admission showed the presence of large con-



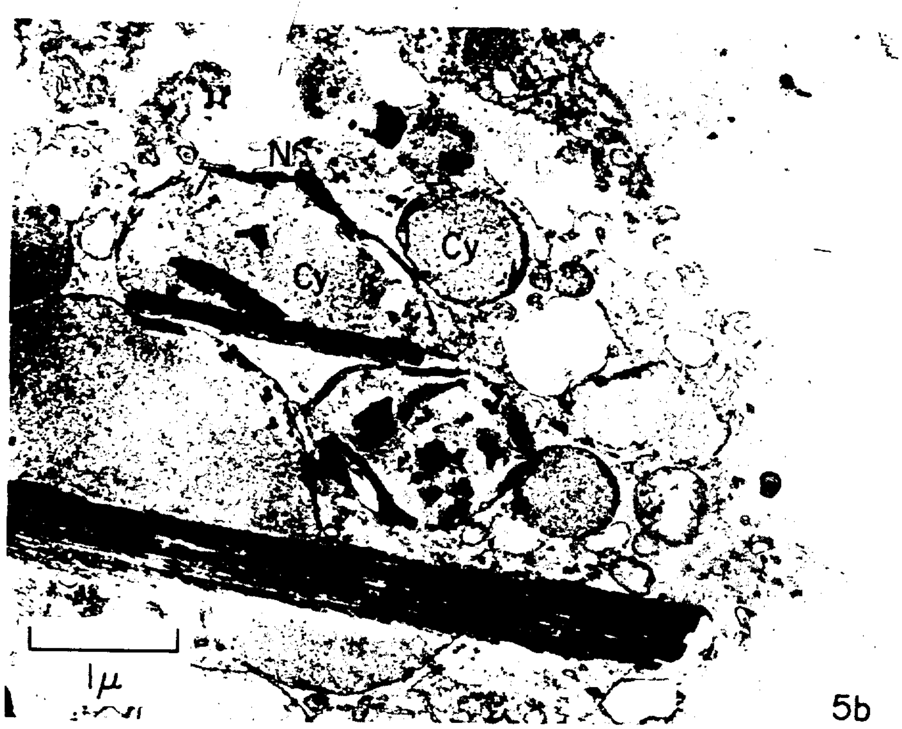


FIGURE 5a. Case 2. Alveolo-capillary wall, showing collagen in basement membrane, disruption of epithelial layer and vesiculation of endothelium. ×6,800.

FIGURE 5b. Case 2. Area containing cytosomes (Cy) lined with needles and two large particles with longitudinal internal structure. ×18,000.

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FIGURE 6b. (
shown. ×13,90

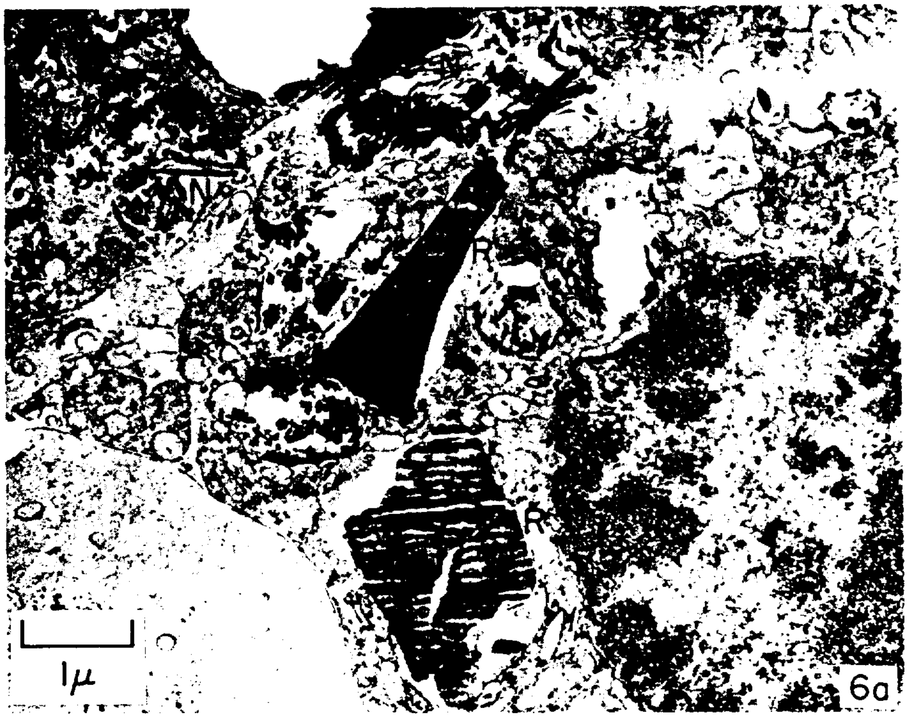

FIGURE 6a. Case 2. Area showing needles and rectangular, chattered material. ×13,900.

FIGURE 6b. Case 2. Cytosomes containing needles. A granular aggregate is also shown. ×13,900.

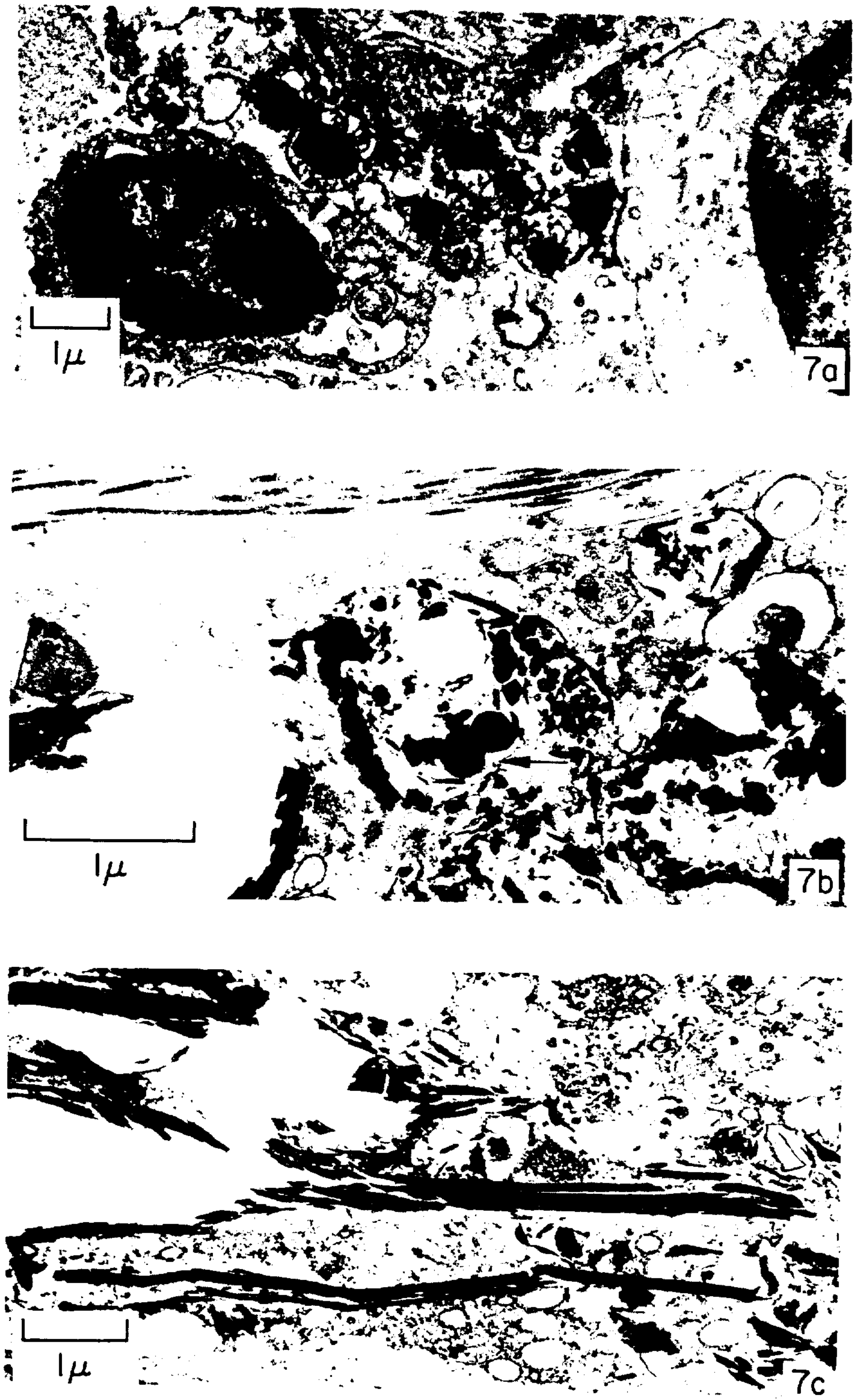

FIGURE 7a. Case 2. Membrane-bound square particles. ×10,400.
FIGURE 7b. Case 2. Spheres, possibly of SiO_2. ×21,900.
FIGURE 7c. Case 3. Long, thin arrays of needle-like material. ×13,700.

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TABLE 2a

PULMONARY FUNCTION DATA FOR SUBJECT 3

Test	Predicted	Observed	Predicted %
Spirometry and Lung Volumes			
Total lung capacity (liters)	5.30	4.90	92
Residual volume (liters)	1.44	1.87	130
RV/TLC × 100%	27	38	—
Forced vital capacity (liters)	3.86	3.03	78
Forced expiratory volume in one second (liters)	3.09	2.33	75
$FEV_{1.0}/FVC \times 100\%$	80	77	—
Maximal voluntary ventilation (liters/min)	135	97	72
Mechanics			
Static compliance (liters/cm H_2O)	0.23 ± 0.06	0.06	—
Recoil pressure at TLC (cm H_2O)	30 ± 7.5	43	—
Airway resistance (cm $H_2O \times liters^{-1} \times seconds^{-1}$)	1.8 ± 0.5	2.3	—

glomerate masses involving the upper zones of both lungs, superimposed on a background of small opacities. This picture was interpreted as showing Stage C complicated on a background of category 2/3 simple coal workers' pneumoconiosis. Pulmonary function studies were performed in 1967; the results are shown in TABLE 2.

This subject's condition gradually deteriorated over the course of the next three years. He was admitted comatose and died of respiratory failure in March, 1971, at which time an autopsy was performed.

Gross examination of the lungs revealed the presence of large, pigmented fibrotic lesions involving most of both upper lobes. Histologic examination revealed the presence of diffuse aggregations of dust nodules, abnormally

TABLE 2b

PULMONARY FUNCTION DATA FOR SUBJECT 3

Test	Rest (air)	Exercise (air)	Rest 100% O_2
Ventilation Studies			
Minute ventilation (liters)	12.74	52.75	—
Oxygen uptake (ml/min)	320	1560	—
Respiratory frequency	20	39	—
Arterial oxygen tension (mm Hg)	84	69	567
Arterial carbon dioxide tension (mm Hg)	31	31	30
Arterial pH	7.49	7.43	7.49
Arterial oxygen saturation (%)	96	93	100
Wasted ventilation (VD/VT)	0.36	0.29	—
Alveolar-Arterial O_2 difference (mm Hg)	20	38	84
Venous admixture (%)	—	—	5.0

thickened remnants of alveolar walls, and focal emphysema in the areas of lung not involved by the large conglomerate, fibrotic masses (FIGURE 2c).

The area preserved for electron microscopy was largely fibrous connective tissue, and no alveolar sacs have been identified so far in the study. Much of the fibrillar material could be identified as collagen. Scattered throughout this connective tissue were cells containing debris, most of which was the needle-like type. The needles were seen lining the cytosomes, in the interior of the cytosomes, and in large arrays of overlapping chunks (FIGURE 8b). These chunks usually tore the sections, leaving large holes in the tissue. Some of the needle-like material appeared as long narrow pieces (6–7 μ long by 0.1 μ wide) (FIGURE 7c). The internal structure of these particles indicated that they were composed of many smaller, needle-like fragments or that they had broken into these fragments, possibly during processing or sectioning or in the course of natural events in the life of the particles.

There were a number of angular, homogeneous particles, most with dense borders as in the case of subject 1 (FIGURE 8b). This type of material seemed less hard than the needles. It usually sectioned smoothly and did not tear the sections. The shape of the particles was variable, ranging from triangular to irregularly rectangular, and sometimes multiangular bits were seen. The interior was most often completely smooth and homogeneous in appearance, although occasionally a little cross-striation appeared.

Rectangular particles containing the characteristic chatter or horizontal banding were seen in this specimen, also (FIGURE 8a). Granular aggregates were scarce and small. They appeared to be cytosomes that contained needle-like material at the periphery and fine dense granular material in the interior (FIGURE 8a).

Case 4

This 42-year-old male worked for 24 years as a boiler repairman at a power-generating station that burned bituminous coal. He was a nonsmoker. He was admitted to West Virginia University Hospital in March, 1970, for replacement of a stenosed mitral valve, which had resulted from rheumatic fever contracted at age 13. His only respiratory symptom was moderate exertional dyspnea that was completely relieved by replacement of the stenosed mitral valve with a ball valve prosthesis. A chest radiograph made at the time of admission for mitral valve surgery revealed cardiac enlargement and some suggestion of enlargement of the left atrium. Although a few small opacities were evident in the lungs, they were insufficient to diagnose definite pneumoconiosis, and the chest film was ultimately classified as category 0/1.

Light microscopy of the lung sections showed mild to moderate thickening of the walls of the pulmonary arterioles. Focal areas of thickening and fibrosis of alveolar septae were noted. Moderate numbers of pigment-laden macrophages were seen within alveolar spaces. In focal areas, small granulomatous lesions showing clusters of epithelioid cells without central necrosis were noted. Surrounding these clusters of epithelioid cells were layers of mononuclear cells (FIGURE 2d).

The preservation of fine structure was far better in this than in the other material because it was obtained at biopsy. The endothelial and epithelial layers of the alveolo-capillary wall appeared intact and devoid of the extensive vesiculation that was seen in the autopsy specimens. The basement membrane, as

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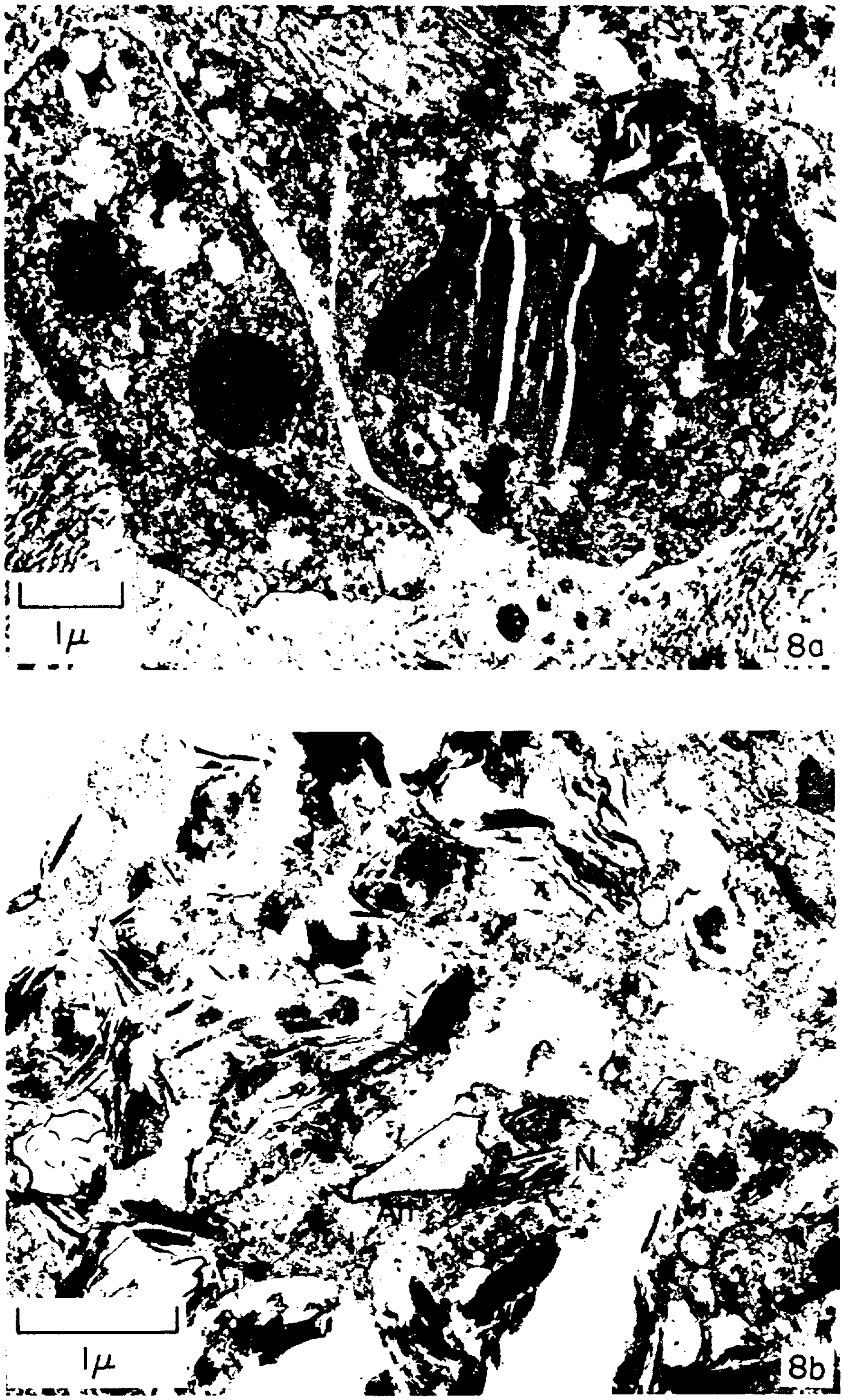

FIGURE 8a. Case 3. A chunk of rectangular material, needles, and a small granular aggregate containing needles. $\times$13,700.

FIGURE 8b. Case 3. Needles and angular pieces with dark borders are most prominent in this section. $\times$20,500.

TABLE 3

PULMONARY FUNCTION DATA FOR SUBJECT 4

Test	Predicted	Observed	% Predicted
Spirometry and Lung Volumes			
Total lung capacity (liters)	6.90	6.28	91
Residual volume (liters)	2.40	2.08	87
RV/TLC 100 (%)	35	33	—
Forced vital capacity (liters)	4.50	4.20	93
Forced expiratory volume in one second (liters)	3.60	3.07	85
$FEV_{1.0}/FVC \times 100$ (%)	80	73	—
Airway resistance (cm $H_2O \times liters^{-1} \times seconds^{-1}$)	1.6 ± 0.5	1.3	—
Diffusing Capacity			
Single breath test ($ml \times mm^{-1} \times mm\ Hg^{-1}$)	37.0	36.0	97

in the other lungs, was much thickened and contained fibrous material and collagen fibers. Occasionally, an extra cell layer was observed in the basement membrane between the epithelial and endothelial cells, making a five-layer "blood-air" barrier, instead of the usual three-layer barrier (FIGURE 9a).

Elastic fibers were prominent in many alveolar septae. Occasionally, these fibers were surrounded by collagen fibers, but this was not the usual finding. Collagen did appear frequently in the alveolar septae (FIGURE 9a) and, as previously mentioned, in the basement membrane of the capillaries. Large whorls of collagen fibers appeared in the interstitial areas (FIGURES 11a & b).

In one alveolar space fibrin was seen in close association with a couple of macrophages (FIGURE 10a). Many spaces contained debris-laden macrophages. Some of the inclusions were the normal ones seen in this type of cell, while others resulted from dust inhalation (FIGURE 10b).

Even though this subject was not an underground coal miner, the particulate matter contained in the lungs was remarkably similar to that found in the other three subjects. There were accumulations of needle-like particles. Some were in cytosomes—either within them or at the periphery—some were free in the cytoplasm, and some were overlapped in groups, forming chunks of material (FIGURES 11a & b).

TABLE 4

TYPES OF PARTICLES FOUND IN LUNGS

Subject	Needles	Angular	Rectangular	Large Aggregates	Round	Square	Asbestos Bodies
1	X	X	X	X			
2	X	X	X	X	X	X	
3	X	X	X	X			
4	X	X	X	X	X		X

FIGURE 9a. (
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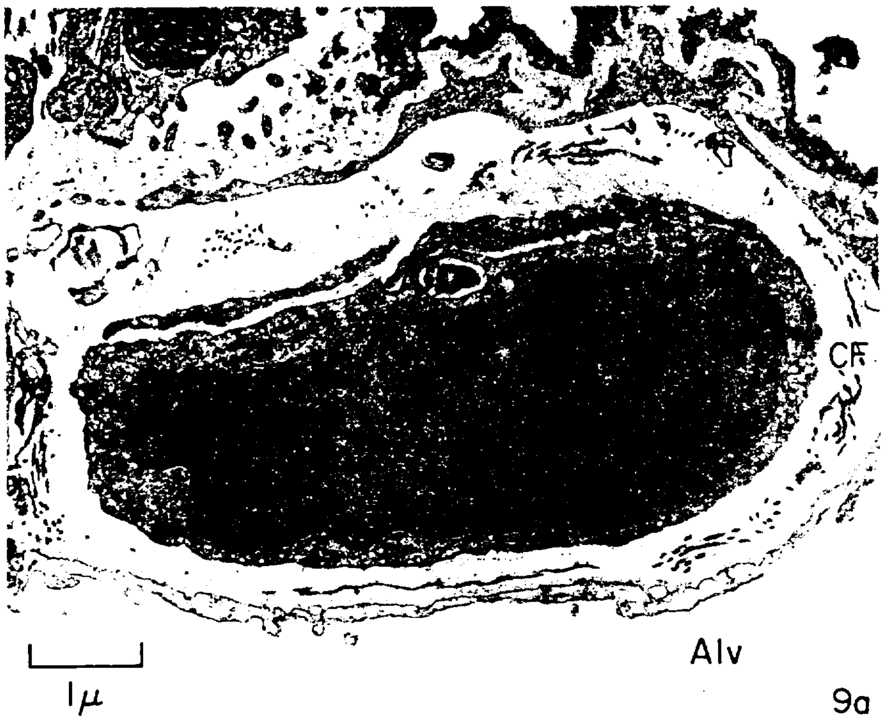
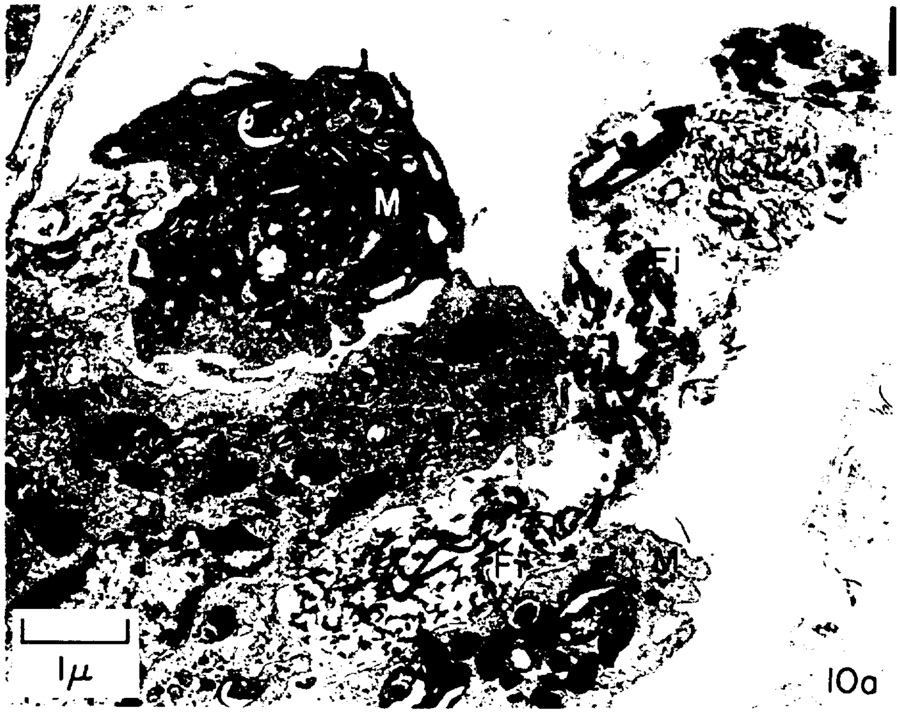


FIGURE 9a. Case 4. Alveolo-capillary wall showing well preserved cells. Basement membrane contains collagen fibers.

FIGURE 9b. Case 4. Asbestos body. Note internal structure and fine granular material surrounding it. ×20,000.



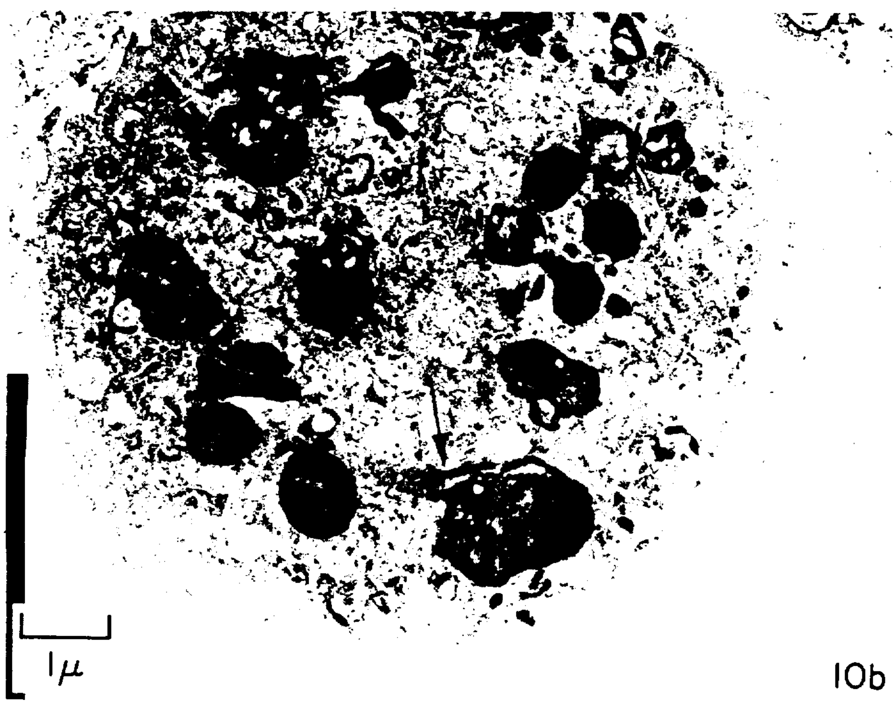


FIGURE 10a. Case 4. Fibrin (Fi) in alveolar space in close proximity to macı phages (M). ×8,000.

FIGURE 10b. Case 4. Alveolar macrophage containing inclusions. Note den hard material (→). ×10,300.

FIGURE 11a. Cas

FIGURE 11b. Case material (→). ×6,

FIGURE 11a. Case 4. All four types of material appear in this section. ×9,400.
FIGURE 11b. Case 4. Unusual aggregate containing a piece of rectangular, chattered material (→). ×6.600.

A number of moderately dense, homogeneous, angular particles with dark borders were observed free in the cytoplasm (FIGURE 11b). Larger cross-striated chunks, the rectangular type, appeared in the same areas (FIGURE 11a). There is a possibility that these two types of material are similar in composition and that the smooth, angular pieces are actually smaller bits of the larger ones. The chatter or cross striation may have occurred in the large particles when the knife was made to traverse a larger, possibly harder area in sectioning.

A number of large, granular aggregates were seen in this tissue. They contained needle-like material, some round particles, and other bits and pieces of debris (FIGURE 11a). A variation on this type of particle appeared as a chunk of the rectangular material coated with a layer of small dense particles (FIGURE 11b).

As in Case 2, this lung also contained small, round debris, possibly silicon dioxide. In addition to these types of particles common to at least one other subject or to all of the subjects, asbestos bodies were noted in a couple of the alveolar macrophages (FIGURE 9b). They were composed of a relatively dense material with a horizontally oriented inner structure surrounded by fine granules, possibly ferritin. They were relatively small for asbestos bodies (about 5 μ by 1 μ) and were wholly enclosed within the macrophage.

SUMMARY

Portions of four lungs were prepared for study by electron microscopy. Three were obtained at autopsy from underground coal miners and one at biopsy from a boiler repairman at a power plant that burned bituminous coal. All four lungs contained large amounts of collagen and elastic fibers. The autopsy specimens showed changes such as membrane disruption and vesiculation, probably resulting from postmortem autolysis.

All four lungs contained a number of particles of various types. Four were common to all of the lungs: (1) hard, dense needle-like material, (2) smooth, angular pieces, (3) larger rectangular chunks with chatter marks, and (4) granular aggregates that often contained some of the other types of particles. In addition, a variety of round and square particles were seen, and in one case asbestos bodies were noted.

Positive identification of the types of particles seen was not possible; thus, their specific role in producing the pathological changes observed is uncertain.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Milton Hales and Dr. Eugene Cassidy of West Virginia University Medical Center, Department of Pathology, Morgantown, West Virginia, for their assistance in providing the light microscopic sections.

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